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COZEN O'CONNOR, P.C.
1900 MARKET STREET
PHILADELPHIA, PA 19103-3508

EXAMINER	
SINGH, ANOOP KUMAR	
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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/692,918	GROSVELD, FRANK	
	Examiner	Art Unit	
	Anoop Singh	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *27 April 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-32* is/are pending in the application.

4a) Of the above claim(s) *17-32* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-16* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☒ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *1/20/04*.

4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

5) ☐ Notice of Informal Patent Application (PTO-152)

6) ☐ Other: _____.

DETAILED ACTION

Claims 1-32 are pending in this application.

Election/Restrictions

Applicant's election with traverse of group I in the response filed dated April 27, 2006 is acknowledged. The traversal is on the ground(s) that Group I and Group II-III should be examined together because search for invention of Group I would be coextensive with Group II and III. In addition, applicants assert that only method of Group I would be required to make the antibody recited in Groups II and III. Applicant's arguments for examining elected method group with the product claims are not persuasive because as recited VHH single chain antibody can be made by another process. For example, Riechmann et al (J Immunol Methods. 1999; 231(1-2): 25-38) describe an intact heavy chain antibody that was readily generated by cloning a particular camel VH in front of the hinge and effector function domains of human IgG1. It is noted that such a constructs in a pcDNA3 vector produced transient expression in COS cells. These chimeric heavy chain antibodies were reported to be fully active in antigen binding (pp 31, col.1, para 2). In addition, it is also disclosed that VHs from camel heavy chain antibodies and the camelised human VH domains have both been successfully fused to g3p and these were found suitable for detection of their antigens in ELISA (pp 30. col. 2, last para bridging to pp 33, col. 1, para 1 and references therein, also see section 6.3).

Accordingly, Riechmann discloses a VHH antibody that would inherently be the same antibody and would not be any different from VHH antibody obtained by any other process.

The requirement is still deemed proper and is therefore made FINAL. Claims 1-16 will be examined in the instant application.

Claims 17-32 have been withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on April 27, 2006.

Claims 1-16 are under consideration.

Priority

Acknowledgment is made of applicant's claim for foreign priority based on an application filed in Great Britain on April 24, 2001. It is noted, however, that applicant has not filed a certified copy of the priority application as required by 35 U.S.C. 119(b).

Drawings

New corrected drawings in compliance with 37 CFR 1.121(d) are required in this application because details on the drawing for human and camel loci are

indistinguishable (see figure 1). Applicant is advised to employ the services of a competent patent draftsperson outside the Office, as the U.S. Patent and Trademark Office no longer prepares new drawings. The corrected drawings are required in reply to the Office action to avoid abandonment of the application. The requirement for corrected drawings will not be held in abeyance.

Specification

The disclosure is objected to because of the following informalities: The disclosure is objected to because it describes Figure 3 in the specification (pp 25, lines 6). However, no such figure is presented.

The disclosure is also objected to because it contains an embedded hyperlink and/or other form of browser-executable code. Applicant is required to delete the embedded hyperlink and/or other form of browser-executable code. See MPEP § 608.01. (For example, see page 21, lines 21).

Appropriate correction is required.

Double Patenting

A rejection based on double patenting of the "same invention" type finds its support in the language of 35 U.S.C. 101 which states that "whoever invents or discovers any new and useful process ... may obtain a patent therefor ..." (Emphasis added). Thus, the term "same invention," in this context, means an invention drawn to identical subject matter. See *Miller v. Eagle Mfg. Co.*, 151 U.S. 186 (1894); *In re Ockert*, 245 F.2d 467, 114 USPQ 330 (CCPA 1957); and *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970).

A statutory type (35 U.S.C. 101) double patenting rejection can be overcome by canceling or amending the conflicting claims so they are no longer coextensive in scope. The filing of a terminal disclaimer cannot overcome a double patenting rejection based upon 35 U.S.C. 101.

Claims 1-16 are provisionally rejected under 35 U.S.C. 101 as claiming the same invention as that of claims 1-16 of copending Application No. 10/693308. This is a provisional double patenting rejection since the conflicting claims have not in fact been patented.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-16 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

In the instant case, claims are directed to a method for the production of a VHH or camelised VH single heavy chain heavy chain antibody in a mammal comprising the step of expressing a heterologous VHH heavy chain locus in that mammal.

In determining whether Applicant's claims are enabled, it must be found that one of skill in the art at the time of invention by applicant would not have had to perform "undue experimentation" to make and/or use the invention claimed. Such a

determination is not a simple factual consideration, but is a conclusion reached by weighing at least eight factors as set forth in In re Wands, 858 F.2d at 737, 8 USPQ 1400, 2d at 1404. Such factors are: (1) The breadth of the claims; (2) The nature of the invention; (3) The state of the art; (4) The level of one of ordinary skill in the art; (5) The level of predictability in the art; (6) The amount of direction and guidance provided by Applicant; (7) The existence of working examples; and (8) The quantity of experimentation needed to make and/or use the invention.

These factors will be analyzed, in turn, to demonstrate that one of ordinary skill in the art would have had to perform "undue experimentation" to make and/or use the invention and therefore, applicant's claims are not enabled.

Furthermore, USPTO does not have laboratory facilities to test if an invention will function as claimed when working example are not disclosed in the specification, therefore enablement issues are raised and discussed based on the state of knowledge pertinent to an art at the time of the invention, therefore, skepticism raised in enablement rejections are those raised in the art by artisan of expertise.

The aspects considered broad are: breadth of subject population for expressing VHH heavy chain locus, any vector for cloning mgabase transgene, using ES cells of any species and expressing VHH locus.

The specification fails to provide an enabling disclosure for the claimed invention because the specification fails to provide sufficient guidance as to (i) how an artisan of skill would have practiced the claimed method in any mammal, (ii) how complex cloning of megabase size single chain antibody locus is cloned. An artisan would have to carry

out extensive experimentation to make and use the invention, and such experimentation would have been undue because art of expressing VHH single amino acid locus in any mammal *in vivo* is unpredictable and specification fails to provide any guidance as to how the claimed method would have been practiced in any mammal. As will be shown below, broad aspects were not enabled for the claimed invention at the time of filing of this application because neither the specification nor the art of record taught sufficient guidance to practice the claimed invention. For purposes to be shown in the state of the prior art, the question of lack of enablement is discussed.

As a first issue, the claims 1-16 embrace for the production of a VHH or camelised VH single heavy chain heavy chain antibody in a mammal comprising the step of expressing a heterologous VHH heavy chain locus in that mammal. The specification contemplates using vector such as YAC or BAC that is suitable of inserting large amounts of nucleic acid, sufficient to encode an entire immunoglobulin heavy chain locus (pp 15, lines 14-23). The specification further contemplates making transgenic mice that is capable of producing fully functional specific single chain VHH antibody which undergoes a process of evolution similar or the same as that of camelid antibodies produced in their native environment. However, the specification provides only prophetic and a general methodology without disclosing any specifics. The specification only provides prophetic reference to most of the method steps without giving any sequence information for the YAC or BACs. The specification also fails to provide any sequence information for the vectors that would be required for multi step cloning process for expressing transgene *in vivo*. The disclosed figure 1 does not

provide sufficient details to enable one skilled in the art to recreate the complex cloning process prophetically taught in the specification for generating VHH single chain antibody locus using any vector, particularly as the sequence and structure of such a BAC or YACs is not known. It is apparent that each method of expressing single chain VHH locus in different subject requires further experimentation that is not routine and subject to variation in anticipated class switching as contemplated by specification.

As a second issue, the scope of invention as claimed encompasses a method for producing VHH single chain antibody in any mammal. It has been difficult to predict the method as contemplated in the specification would result in functional VHH antibody. For example, De Genst et al (Dev Comp Immunol. 2006; 30(1-2): 187-98) in a post filing art while reviewing the state of antibody repertoire development in camelids state "The humoral immune response of the *Camelidae* is unique as these animals posses the heavy-chain of antibodies that lack the L-chain, and it was noticed that their H-chain is devoid of the typical first constant domain (CH1) and contains a dedicated variable domain VHH. The VHH exon is assembled from separate V–D–J gene segments. The recombined VHH region is subjected to somatic hyper mutations; however, the timing and actual mechanism of the <u>class switch</u> from μ to the dedicated γ-isotype remains elusive" (abstract). It is noted that Genst describes that "the homology with the human/mouse situation, it is assumed that the successful recombination of a VH germline gene with a D and a JH germline minigene leads to the expression of a μ-chain associated with the VpreB and λ5 surrogate chains on the pre-B cell membrane."... Genst further states "The situation is far more problematic whenever one

of the VHH germline genes recombines with a D–JH assembled product in the pre-B cell. In this event, it remains obscure how such an expressed μ-chain could associate with the surrogate L-chain partners. According to the current idea, the combined VpreB and λ5 complex replaces the BiP chaperon proteins on the H-chain to overcome their retention in the endoplasmic reticulum. The associated VHH domain will supposedly resist VpreB pairing, whereas the CH1 of μ requires the removal of the BiP and concomitant λ5 association for the B cell-membrane display of the H-chain. Hence, it is difficult to envisage the expression of a μ-chain carrying a VHH domain on B cells, since the BiP interacting with the CH1 domain will inhibit transport of the μ-chain to the membrane of the B-cell. Therefore, it might be that cells with a properly recombined VHH-D-J gene associated with a μ chain will fail to start V–JL recombination, but will proceed to a class switch to one of the dedicated HCAb γ genes, possibly by an antigen-independent mechanism-although this remains speculative at the current stage of knowledge (pp 194, col. 1, para 2 bridging to col. 2, para 1). The teaching of Genst clearly suggest that mechanism of pre B cell maturation for the production of VHH single heavy chain was not known rather speculative at the time of filing of this application. It is also not apparent from the specification whether a method as recited in claims would result in fully functional scIgG molecule. The specification fails to provide an enabling disclosure for the claimed invention because the specification fails to provide sufficient guidance or any example as to how an artisan of skill would have practiced the claimed method in any mammal by a VH heavy chain locus capable of generating other iso forms of antibodies. An artisan would have to carry out extensive

experimentation to make and use the invention, and such experimentation would have been undue because art of the B cell maturation of VHH camelid antibody was not routine rather it was unpredictable and specification fails to provide any guidance as to how the claimed method would have been practiced.

As a third tissue, the specification contemplates using VHH or camelised VH single heavy chain. However, prior art suggest that repertoires of camelised VH domains were initially created by randomization of residues within the third hypervariable loop H3, which at the same time was varied in length. Riechmann et al (J Immunol Methods. 1999; 231(1-2): 25-38) while reviewing the state of the art in preparing camelised VHH state "the three camelising mutations (G44E, L45R and W47G), which increased the solubility of the single human VH domain most and lead to the least nonspecific binding of phage displayed VH domains, compromised the stability and most significantly the expression yield of active protein for the resulting soluble VH" (pp 30, col. 1, para 3). Riechmann notes "..camelised human VH domains may need additional modifications in their now exposed, former VL interface to tolerate the exposure to a hydrophilic surrounding while maintaining a high stability and good folding properties and providing a highly diverse antigen binding site (pp 35, col.2, para 1)". The teaching of Riechmann et al suggest that more refinement in camelised VH domain was required to maintain stability and folding properties at the time of filing of this application. The specification does not provide any guidance to overcome this art recognized limitation. An artisan would have to carry out extensive experimentation to make use the invention, and such experimentation would have been undue because of

the art recognized unpredictability and specification fails to provide any guidance as to how the claimed method would have been practiced.

As a final issue, the claims 1-16 embrace a method for producing a VHH single chain antibody by expressing a heterologous VHH heavy chain locus in that mammal. The specification asserts loci and vectors may be introduced into an animal to produce a transgenic animal. It is noted that method of inserting the loci into the genome of a recipient animal will be achieved by microinjection or by introducing DNA into embryonic stem cells (ES) cells which can be inserted into a host embryo to derive transgenic mice (pp 27, lines 21-31 bridging to pp 28 see entire section). The state of prior art summarized by Cameron (Molecular Biotechnology 7: 253-265) describes that the art of making a transgenic nonhuman animal is not predictable because of several factors. It is noted, " Well regulated transgene expression is the key to successful transgenic work, but all too often experiments are blighted by poor levels or complete absence of expression, as well as less common problems, such as leaky expression in non targeted tissues. A feature common to any transgenic experiments is unpredictable transgenic lines produced with same construct frequently displaying different levels of expression. Further, expression levels do not correlate with number of transgene copies integrated. Such copy number independent expression pattern emphasizes the influence of surrounding chromotin on the transgene" (pp 256; section 4 on transgene regulation and expression). Furthermore Kolbe et al also describe "the expression of foreign gene in transgenic animals is generally unpredictable as transgenes integrated

at random after pro nuclear injection into fertilized oocytes" because of inhibition of neighboring chromatin (Kolb et al, 1999, Gene 227, 21-31, abstract).

In addition, the method of transgenic nonhuman animal, such as those employed in the instant specification requires embryonic stem cell. Houdebine et al (Journal of Biotechnology, 1994, Vol., 34, pp 269-287) describe that although ES cells can be used to generate transgenic animals, but this approach remains restricted to mice, ES cells from other species are not presently available (pp 279). In addition, Mullin et al also point that non-mouse ES cell capable of providing germ line chimeras were not available (Mullins et al., Journal of Clinical Investigation, 1996, pp 1557, 1st paragraph). Thus, the state of the art is such that ES cell technology is generally limited to the mouse system and that only putative ES cells exist for other species (Moreadith et al., J. Mol. Med., 1997 p214, abstract, Hochepied et al (Stem Cells, 2004, 22, 441-447; abstract). Thus, in view of the prior art and lack of guidance provided in the specification, only mouse ES cells would be enabled to produce any transgenic mice.

The cited arts clearly indicate an unpredictable status of the class switching art pertaining to recombined VHH region assembled from separate V-D-J gene segments. In conclusion, in view of breadth of the claims and absence of a strong showing by Applicant, in the way of specific guidance and direction, and/or working examples demonstrating the same, such invention as claimed by Applicant is not enabled for the claimed inventions. The specification and prior art do not teach a method of producing VHH single chain antibody in a mammal by expressing heterologous recombined VHH region assembled from separate V-D-J gene segments as recited in claims. An artisan

of skill would have required undue experimentation to develop/design a suitable vector and practice the method as claimed because the art of class switching and vector design was unpredictable at the time of filing of this application as supported by the observations in the art record.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-2 are rejected under 35 U.S.C. 102(b) as being anticipated by Ledbetter et al (WO 99/42077, dated 08/26/1999, IDS).

Ledbetter et al teach expressing VHH gene sequence in animals by transgenic teachnology to make founder animals that expresses llama VHH (pp 32, lines 4-5). Further, Ledbetter contemplates using retroviral or pronuclear microinjection of gene targeting in ES cells to produce transgenic clones containing VHH transgene (pp 32, para. 3, lines 15-23). In addition, Ledbetter also describes a multi specific molecule that may be produced by recombinant expression of polynucleotide that encodes these polypeptides that are single chain polypeptide. Ledbetter et al also disclose that camels secrets antibodies devoid of light chain. It is noted that Ledbetter also teaches that the variable region of such heavy chain VHH are fused directly to a hinge region, which is

linked to the CH2 and CH3 domains (pp 18, lines 2-4). Since Ledbetter taught a functional VHH single chain antibody expressing scIgG in a transgenic mice it would inherently contain D and J exon along with constant heavy chain as recited in claim 2.

Accordingly, Ledbetter anticipates claims 1-2.

Claim Rejections - 35 USC § 103

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Claims 1-4, 7-10 and 14-16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lonberg et al (US Patent no 5874299, dated 2/23/1999) and Riechmann et al (J Immunol Methods. 1999; 231(1-2): 25-38.

Lonbeg et al teach a methods to generate a synthetic immunoglobulin variable region gene segment repertoire used in transgene construction and a methods to induce heterologous antibody production using animals containing heterologous rearranged or unrearranged heavy and light chain immunoglobulin transgenes (abstract). It is noted that heterologous heavy or light unrearranged immunoglobulin

transgenes are introduced into a host non-human animal to produce a transgenic non-human animal containing a heavy and a light heterologous immunoglobulin gene (col. 4, lines 3-5). The process of joining of various immunoglobulin gene segments, the V, D, J and constant (C) gene segments are disclosed (col.8, lines 50-65 bridging to col. 9, lines 1-35). The method also comprises a transgenes comprising rearranged heavy and/or light immunoglobulin transgenes. Specific segments of such transgenes corresponding to functionally rearranged VDJ or VJ segments, contain immunoglobulin DNA sequences which are also clearly distinguishable from the endogenous immunoglobulin gene segments in the mouse (col. 11, lines 64 bridging to col. 12, lines 1-3). Lonberg et al also teach that the heavy chain transgene contains more than one C region gene segment such as Cμ and Cγ from the human genome, as a switch regions that are incorporated upstream from each of the constant region gene segments and downstream from the variable region gene segments to permit recombination between such constant regions to allow for immunoglobulin class switching, e.g. from IgM to IgG. Such immunoglobulin transgenes also contain transcription control sequences including promoter regions situated upstream from the variable region gene segments which contain OCTA and TATA motifs (col. 13, lines 16-26). It is noted that a transgenic mouse line containing this transgene correctly expresses all of the heavy chain classes required for B-cell development as well as a large enough repertoire of variable regions to trigger a secondary response for most antigens (col. 17, lines 20-30). Furthermore, Lonberg et al contemplate immunoglobulin heavy and light chain transgenes comprises one or more of each of the V, D, J and C gene segments contain at least one μ gene

and gamma gene segment of constant region gene segment (col. 18, lines 53-57). In addition, Lonberg contemplate using leader signal sequence to effect secretion through and/or insertion of the immunoglobulin into the transmembrane region of the B-cell (col. 9, lines 51-60). However, Loneberg does not specifically disclose a transgene wherein VH region comprises a camel VH region or camelised VH region.

Prior to the invention, Riechmann et al teach a method to prepare and obtain recombinant forms of camel VH domain and camelised VH domain (and references therein). Riechmann teaches that camelised VH domains are synthetic and therefore these are generated in vitro. It is noted that the human VH3 domain with the camelised VL interface (with mutated residues 44, 45 and 47) is used as a building block for the creation of designed libraries (pp 29, col. 2, lines 1-10). Riechmann et al disclose that VH repertoires can be created through the introduction of randomized regions into the VH gene to vary the hypervariable loops both in length and amino acid residue. It is also disclosed that camelised VH domains present a useful building block for the preparation of Ig-based recognition units of minimal size (see pp 29 col. 2 and pp 30. col 1). Riechmann describes advantages of camelised, human VHs compared to camel VH domains that includes the presence of a Protein A binding in the case of domains based on the human VH3 gene family and potential of using camelised, human VHs for therapeutic purpose in humans due to a lower immunogenicity. However, Riechmann et al do not teach a method for the production of VHH/camelised VH single chain antibody in a mammal.

Thus, based upon motivation for using a camelised VHH or VHH sequence taught by Riechmann and in view of detailed guidance provided in the Loneberg specification for switch sequence, constant region sequence, cloning methodology and method for generating heterologous antibody in a nonhuman mammal, it would have been prima facie obvious to the skilled artisan to modify the specific transgene human VH taught by Lonberg to camel VH or camelised VH domains disclosed by Riechmann. In addition, Riechmann provided motivation by suggesting that camelised human VHs could be used for therapeutic purpose in humans due to lower immunogenicity. The skilled artisan would have been motivated to produce heterologous antibody in a nonhuman mammal by further optimizing and changing D and J region from other species.

Further in view of the high level of skill in molecular biology techniques at the time of filing, one of the ordinary skills in the art would expect a reasonable expectation of success in modifying the transgene to include VHH/camelised VH region in the method disclosed by Lonberg for producing heterologous antibody in mouse. Lonberg had already described switch sequence, constant region sequence, cloning methodology and method for generating heterologous antibody in a nonhuman mammal and it would have only required routine experimentation to replace human VH to VHH disclosed by Riechmann. One of ordinary skill in art would have been motivated to combine the teaching of Lonberg and Riechmann because a method to generate VHH or camelised VH single heavy chain heavy chain antibody in a mammal would have yielded a single chain camelised VHH antibody for therapeutic use in humans.

Claims 1-4, 7-16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lonberg et al (US Patent no 5874299, dated 2/23/1999) and Riechmann et al (J Immunol Methods. 1999; 231(1-2): 25-38 and further in view of Lonberg et al (US Patent no 5625126, dated 4/29/1997).

The combined teachings of Lonberg ('299) and Riechmann have been discussed above. However, none of the references explicitly teaches constant heavy region from other species.

Lonbeg et al ('126) teach a method to induce heterologous antibody production of various isotypes, including: IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgA, IgD, of IgE (col. 4, line 9-10) in the transgenic non-human animal (col. 2, lines 59-60). Lonberg also disclose mice that produce B cells is also capable of alternatively expressing antibodies comprising fully human heavy chains and antibodies comprising chimeric (human variable/mouse constant) heavy chains, by trans-switching (col. 43, lines 3-45). Lonberg discloses that the immunoglobulin heavy chain transgene could comprise one or more of each of the VH, D, and JH gene segments and two or more of the CH genes. It is noted that with regard to the CH segments for the heavy chain transgene, it is preferred that the transgene contain at least one μgene segment and at least one other constant region gene segment, more preferably a γ gene segment. Lonberg discloses that switch regions can be linked upstream heavy chain C gene that do not naturally occurs next to a particular switch region (col. 33, lines 1-6). Lonberg also discloses that murine γ2a and γ2b can also be used, as may downstream (i.e., switched) isotype genes form

various other species (col. 34, lines 19-24). Lonberg teaches that the switch regions used in the transgene are preferably murine or human (col. 33, lines 62-67). Lonberg provide motivation for using a murine rather than a human switch region in combination with human constant region gene by teaching that switch sequence derived from those which occur naturally in the mouse that is to receive transgene, may produce a higher frequency of isotype switching events as the mouse switch sequence are optimized to function with the mouse switch recombinase enzyme system (col 25, lines 49-57). However, Loneberg ('126) does not specifically disclose a transgene wherein VH region comprises a camel VH region or camelised VH region.

Thus, based upon motivation for using a camelised VH or VHH sequence taught by Riechmann and in view of detailed guidance provided in the Loneberg (299') specification for switch sequence, constant region sequence, cloning methodology and method for generating heterologous antibody in a nonhuman mammal, it would have been prima facie obvious to the skilled artisan to modify the specific transgene comprising human VH taught by Lonberg to camel VH or camelised VH domains disclosed by Riechmann. In addition, Riechmann provided motivation by suggesting that camelised human VHs could be used for therapeutic purpose in humans due to lower immunogenicity. The skilled artisan would have been motivated to produce heterologous antibody in a nonhuman mammal by further optimizing and changing constant heavy chain gene from other species such as mouse and rabbit. Lonberg ('126) had already disclosed that switch region from different isotype can be operatively linked to a particular constant region, the skilled artisan would have been motivated to

use either a switch region of same isotype or a switch region of different isotype to promote class switch to any particular constant region gene. In addition, Lonberg ('126) provided motivation for using murine switch sequence to control class switch of human constant regions and further in view of detailed teaching of Lonberg ('299) as discusses above, it would have been *prima facie* obvious to the skilled artisan at the time of filing to modify the switch region of different species as taught by Lonberg ('126). The skilled artisan would have been further motivated to produce heterologous antibody in a nonhuman mammal by further optimizing and changing D and J region from other species.

Further in view of the high level of skill in molecular biology techniques at the time of filing, one of the ordinary skills in the art would expect a reasonable expectation of success in modifying the switch regions from different species and transgene to include VHH/camelised VH region in the method disclosed by Lonberg ('126) for producing heterologous antibody in mouse taught by Lonberg ('299). Lonberg ('299) had already described switch sequence, constant region sequence, cloning methodology and method for generating heterologous antibody in a nonhuman mammal and it would have only required routine experimentation to replace human VH to VHH disclosed by Riechmann. One of ordinary skill in art would have been motivated to combine the teaching of Lonberg ('299), Riechmann and Lonberg ('126) because a method to generate VHH or camelised VH single heavy chain heavy chain antibody in a mammal would have yielded a single chain camelised VHH antibody for therapeutic use in humans.

Claims 1-10 and 14-16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lonberg et al (US Patent no 5874299, dated 2/23/1999); Riechmann et al (J Immunol Methods. 1999; 231(1-2): 25-38 and further in view of Green et al (US Patent Application no 20030093820, dated 11/30/2001, effective filing date 6/8/2000).

The combined teachings of Lonberg and Riechmann have been discussed above. However, none of the references explicitly teaches advantage of using loxP sites.

Green et al teach a method to fully generate human antibodies in a transgenic animal of a desired isotype in response to immunization with any antigen. The human immunoglobulin heavy chain transgene in the foregoing animals comprises a human constant region gene segment comprising exons encoding the desired heavy chain isotype, operably linked to switch segments from a constant region of a different heavy chain isotype, i.e., a non-cognate switch region (abstract). In addition, Green et al also contemplate that the transgenes comprising non-cognate switch region may be a switch region from a different species than the constant region-coding segment. Green et al also teach to use recombinase and specifically use the insertion of lox-P sites into the DNA of a yH YAC (pp 9 para 84). It is note that Green et al disclose that once hybridomas are generated from the transgenic mouse carrying the yH transgene engineered with the lxoP site, CRE-lox mediate class switching can be induced by co-transfecting to get desired mAb (pp 9 para 86). However Green et al do not disclose VHH single chain or camelised single chain antibody.

Thus, based upon motivation for using a camelised VHH or VHH sequence taught by Riechmann and in view of detailed guidance provided in the Loneberg specification for switch sequence, constant region sequence, cloning methodology and method for generating heterologous antibody in a nonhuman mammal, it would have been prima facie obvious to the skilled artisan to modify the specific transgene human VH taught by Lonberg to camel VH or camelised VH domains disclosed by Riechmann. In addition, Riechmann provided motivation by suggesting that camelised human VHs could be used for therapeutic purpose in humans due to lower immunogenicity. The skilled artisan would have been further motivated to introduce loxP sites or any other known recombinases system such as Flp in the transgene as Green et al had already described that one could separate desired antibody from the hybridoma (eg IgM) by Cre mediated excision.

Further in view of the high level of skill in molecular biology techniques at the time of filing, one of the ordinary skills in the art would expect a reasonable expectation of success in modifying the transgene to include VHH/camelised VH region and introduce lox/flp in the method disclosed by Lonberg for producing heterologous antibody in mouse. Furthermore, Lonberg had already described switch sequence, constant region sequence, cloning methodology and method for generating heterologous antibody in a nonhuman mammal and it would have only required routine experimentation to replace human VH to VHH disclosed by Riechmann or introduce loxP sites in the transgene as suggested by Green.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Conclusion

No claims allowed

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure:

Lonberg et al (US Patent No. 5569825, dated 10/29/1996)

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Anoop Singh whose telephone number is (571) 272-3306. The examiner can normally be reached on 9:00AM-5:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached on (571) 272- 0735. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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Anoop Singh, Ph.D.
Examiner, 1632

Joe Woitach
AU1632